Clin Exp Immunol 1995; **102**:224–228

Genetic variation in neutrophil accumulation in mice is not mediated through immigrant regulatory cells

S. B. MARLEY, C. L. HADLEY & D. WAKELIN *Department of Life Science, University of Nottingham, Nottingham, UK*

(Accepted for publication 19 May 1995)

SUMMARY

Genetic variation of induced peritoneal neutrophilia in mice was accompanied by parallel variation in macrophage responses. The timing of the macrophage responses in high responder (C57Bl/10) mice indicated a potential role for these cells in mediating the enhanced neutrophil response. However, adoptive transfer of inflammatory macrophages did not induce neutrophilia. Analysis of peritoneal cytokine levels in high and low responder mice further indicated that IL-1, IL-3, GM-CSF, G-CSF and interferon-gamma (IFN-γ) were not involved in mediating the genetic variation observed. Exogenous tumour necrosis factor-alpha (TNF-α) was effective in inducing the high responder phenotype, despite the absence of detectable TNF-α in either peritoneal fluid or serum. A role for genetically determined differential expression of endothelial adhesion molecules in high and low responders is suggested.

Keywords neutrophilia genetic control macrophage TNF

INTRODUCTION

Neutrophil accumulation and function varies widely in normal individuals as well as in those with specific genetic defects or aquired pathological abnormalities [1,2]. Experimental evidence of genetic variation is available from several species, including rats [3] and cattle [4] but most notably in mice (e.g. [5–7]).

We have described [8] a murine model of genetic variation in neutrophil accumulation. Mice challenged with intra-peritoneal injection of a sterile irritant (Brewer thioglycollate) behaved as low (BALB/c) or high responders (C57Bl/10 [B.10]). LPS was implicated as the specific trigger. The high responder (HR) phenotype was dominant in F_1 progeny and largely independent of MHC expression. Neutrophil accumulation was comparable up to 9 h after injection, reaching a plateau at this point in BALB/c mice, but continued in B.10 mice up to 15–18 h after injection. No significant peripheral or marrow neutropaenia was observed, suggesting that variation in the duration of neutrophil accumulation was not due to a depletion of reserves.

Neutrophil production is influenced by a series of cytokines [9]. Cells released from the marrow circulate briefly in the blood before migration into tissues. Endothelial adhesion and extravasation are effected via adhesion molecule/ligand interactions and up-regulated by pro-inflammatory cytokines (e.g. IL-1,TNF) released following an inflammatory stimulus [10]. The process is enhanced by vascular changes induced by mediators released from regulatory cells responding either to the initial stimulus or to the subsequent cytokine release. Migration is along gradients of locally produced chemotactic factors.

Correspondence: D. Wakelin, Department of Life Science, University of Nottingham, University Park, Nottingham NG7 2RD, UK.

Candidate regulatory cells within the peritoneal cavity include T cells, macrophages and mast cells. T cells are not directly sensitive to LPS but can respond to signals from LPS-sensitive cells to release cytokines that influence production and accumulation of neutrophils and macrophages [11–13]. Peritoneal macrophages stimulated *in vitro* with LPS, or with mediators from other cells, release a broad range of inflammatory and colony-stimulating factors that can increase neutrophil production and activation [14]. Mast cells similarly may be triggered to release a variety of mediators [15,16]. Genetic variation in neutrophil accumulation could therefore be mediated by variation in the response of any of these regulatory cell populations to LPS stimulation.

MATERIALS AND METHODS

Mice

SPF female BALB/c and C57Bl/10 mice from Harlan-Olac (Bicester, Oxon, UK) were used at 5–6 weeks old. Responses at this age are similar to those in older mice (unpublished data). (BALB/c × C57Bl/10) F_1 hybrids were bred in-house.

Induction of peritoneal inflammatory responses

Brewer thioglycollate (BT). Mice were injected intraperitoneally (i.p.) with 1 ml sterile 3% BT (Difco, East Molesey, UK).

Bacteria. Staphylococcus aureus, Staph. epidermidis and *Escherichia coli* (NM522) were inactivated in 4% formaldehyde, washed in sterile, pyrogen-free saline and stored at −80°C. Mice were injected i.p. with 1 ml volumes at concentrations ranging from 104 to 1010 colony forming units (CFU)/ml.

LPS. Mice were injected i.p. with 1 ml of LPS (*E. coli* serotype 0111:B4, Sigma Chemicals, Poole, UK) in sterile saline, at concentrations up to 2500 μg/ml.

IL-1-α and TNF-α. Mice were injected i.p. with 100 ng IL-1α (AMS) and 500 ng LPS-free TNF-α (NBS Biologicals, Hatfield, UK).

Harvesting and counting peritoneal cells

Non-adherent cells. Mice were killed and peritoneal cells harvested immediately by injection of 4 ml RPMI (Gibco, Paisley, UK), gentle abdominal massage for 30 s and withdrawal of 3·5 ml of fluid. All samples were taken between 09.00 h and 11.00 h. Cells were counted by haemocytometer and their viability assessed by metabolism of 10 ng/ml fluorescein diacetate (Sigma). Cytospin preparations, fixed in methanol, were stained with Wright's stain for differential counts (on at least 200 cells). As responses in sham-inoculated animals (1 ml pyrogen-free saline i.p.) were not statistically different (data not shown) from untreated animals at 18 h post-injection (p.i.), untreated animals were used as controls.

Adherent cells. Non-adherent peritoneal cells were removed by lavage with Ca/Mg-free Hanks Buffer (Gibco). The peritoneal cavity was flushed again with 4 ml buffer, drained and injected with 4 ml warm 0·25% trypsin (Gibco) in PBS. After 10 min at 37°C, the trypsin solution was withdrawn and forcibly reinjected to detach loose cells, 2 ml were withdrawn and mixed with 2 ml ice-cold RPMI + 10% FCS. Cells present in the initial lavage and the trypsin/RPMI solution were then counted.

Measurement of cytokines

The peritoneal cavity was flushed with a 0·5 ml chilled 10% FCS in RPMI, the fluid centrifuged and the supernatant removed. Samples were used directly (for IL-1, IFN-γ, IL-3, TNF-α) or stored at −80°C (for GM-CSF, G-CSF). Blood was collected over 0·5 ml Sera-sieve, allowed to clot at 4°C and centrifuged. Sera were assayed immediately. Concentrations of GM-CSF, IL-3, IFN-γ and TNF-α were measured by conventional ELISA using matched antibody pairs (Pharmingen, San Diego, CA). The standards used were TNF (NBS Biologicals, UK), IL-3 (Genzyme, UK), GM-CSF (Serotec, UK), IFN-γ (Dr K. Robinson, Life Science).

IL-1α was measured by commercial ELISA kit (Intertest-1α, Genzyme, USA) and G-CSF measured biologically [17]. NSF60 cells were dispensed into eight duplicate wells of a 96-well plate each containing 50 μl of a 1:2 dilution series of either G-CSF standard or test sample in RPMI + 10% FCS. Ten μl each of anti-IL-3 and anti-GM-CSF were added to four of the eight wells. After incubation, 0·25 mCi of ^{3}H-thymidine (Amersham International, Aylesbury, UK) was added per well, the cells harvested and activity counted on a Packard 1900TR scintillation counter.

Adoptive transfer

Peritoneal cells from control B.10 or BT-injected donors were resuspended in 1 ml and injected directly into homologous (B.10) recipients (1:1 ratio). Peritoneal neutrophilia was assessed 18 h later. Results are given as the means of three recipients.

Statistical analysis

Data were analysed using the Mann–Whitney *U*-test. Values of $P < 0{\cdot}05$ were considered significant.

RESULTS

Peritoneal inflammatory cell kinetics after injection of BT (Fig. 1a–d)

In both strains neutrophils increased significantly within 3 h reaching maximum levels in BALB/c mice at 9 h p.i. but accumulating in B.10 until 15 h p.i. Numbers were significantly greater in B.10 mice from 12 to 24 h p.i. Lymphocytes fluctuated during the first 18 h, with a significant increase only in B.10 mice but rising in both strains between 18 and 24 h. Inter-strain differences were significant only at 9 h p.i. BALB/c mice had significantly greater resting levels of mast cells ($P < 0{\cdot}05$) than B.10 mice, but both strains then showed a rapid decline. From 9 to 24 h p.i. mast cells were barely detectable in B.10 mice; in BALB/c there were significant numbers at 9, 12 and 18 h p.i. ($P < 0{\cdot}01$). Peritoneal macrophages (Fig. 1d) showed a slight reduction in both strains p.i., followed by a significant increase. The onset of the increase was different for the two strains, starting after 9 h p.i. in B.10 mice and 18 h p.i. in BALB/c. The proportion of cells with reduced cytoplasmic volume and vacuolation increased markedly in BT-stimulated animals, suggesting the immigration of blood monocytes.

Cytokine profiles in the peritoneal cavity (Table 1)

Neither GM-CSF nor TNF-α were detectable in either strain at any time. TNF-α was also not detected in serum samples. IL-3, IFN-γ, IL-1α and G-CSF all increased 3–6 h p.i. in both strains but declined to near baseline levels by 24 h. The increase was minimal for IL-3 and IFN-γ, but IL-1α and G-CSF each showed highly significant increases ($P < 0{\cdot}05$) at 3, 6 and 9 h p.i. (both strains) and at 12 and 18 h p.i. (BALB/c). Strain-related differences were apparent for IFN-γ and IL-3 (3 h) and G-CSF (6 h). At both times cytokine levels were higher in BALB/c ($P < 0{\cdot}05$). (G-CSF values were measured with neutralizing antibodies to IL-3 and GM-CSF.)

Effect of BT on peritoneal macrophage adherence (Table 2)

The proportion of adherent macrophages rose from 55% to 68% in BALB/c and from 13% to 37% in B.10 mice. Numbers adherent at 18 h p.i. did not exceed the adherent and non-adherent total recorded prior to injection. Few neutrophils were present in unstimulated animals, making comparison with stimulated animals unreliable. However, in contrast to macrophages, the numbers of adherent neutrophils in stimulated animals were greatly increased and cannot be accounted for by adherence of the resident population following injection of BT.

Expression of high-response phenotype for neutrophils and macrophages by B.10 and F_1 mice

Peritoneal cell numbers were compared between the strains and

Table 1. Cytokine levels during the peritoneal inflammatory response

	Time (h)						
	0	3	6	9	12	18	24
IFN-γ (μ/ml)							
BALB/c	1·3 ± 1·9	10·2 ± 6·4	2·2 ± 2·2	2·8 ± 2·8	2·7 ± 1·8	<1·0	1·7 ± 1·7
B.10	2·0 ± 0·7	3·2 ± 3·2	4·8 ± 1·5	1·2 ± 0·8	3·2 ± 0·8	1·3 ± 1·2	<1·0
IL-1α (pg/ml)							
BALB/c	<10	44·0 ± 31·7	17·7 ± 2·9	14·2 ± 2·9	12·5 ± 7·0	ND	ND
B.10	<10	51·7 ± 17·2	29·2 ± 21	14·5 ± 7·7	12·2 ± 8·8	ND	ND
IL-3 (μ/ml)							
BALB/c	0·5 ± 0·1	3·0 ± 1·0	1·5 ± 0·7	1·9 ± 0·4	0·9 ± 0·4	0·5 ± 0·3	0·8 ± 0·5
B.10	0·3 ± 0·3	0·3 ± 0·3	1·6 ± 0·3	0·5 ± 0·2	0·9 ± 0·1	0·7 ± 0·2	<0·2
G-CSF* (μ/ml)							
BALB/c	<10	6905 ± 5650	10 647 ± 4352	4431 ± 4258	342 ± 114	58 ± 29	30 ± 15
B.10	<10	5360 ± 4640	1147 ± 752	1345 ± 350	18 ± 3	<10	<10

Cytokine levels in peritoneal exudate fluid were determined by sandwich ELISA or by bioassay (G-CSF only). Fluid from each of three mice was assayed at intervals following injection of BT. Results are expressed as means ± 1 s.d.

* G-CSF bioassay in NFS-60 cells. Counts in wells not treated with neutralizing antibodies to GM-CSF and IL-3 were consistently 10–20% higher than treated controls at all time points.

TNF-α was tested in both peritoneal exudate fluid and serum. TNF-α and GM-CSF were not detected (<1 ng/ml) at any time point.

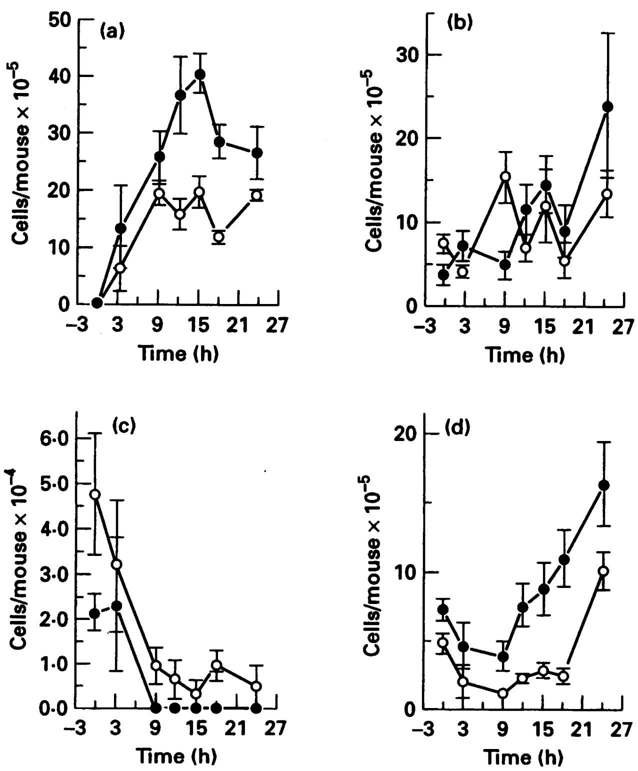

Fig. 1. Kinetics of peritoneal inflammatory response in C57Bl/10 (●) and BALB/c (○) mice after injection of 1 ml sterile Brewer thioglycollate. At intervals, mice were killed, peritoneal cells removed by lavage and absolute numbers determined by haemocytometer. Numbers of (a) neutrophils, (b) lymphocytes, (c) mast cells, and (d) macrophages were then calculated from stained cytocentrifuge preparations. Values shown are the means ± s.e. of five or six mice/time interval.

in F_1 (B.10 × BALB/c) 18 h after various stimuli. Responses in BALB/c were within the ranges previously determined [8], i.e. the LR phenotype was expressed. The response of B.10 mice was identified as LR if not significantly different from BALB/c or HR if significantly greater. After LPS-derived stimulation (BT, *E. coli*, purified LPS) B.10 mice expressed the HR

Table 2. Effect of Brewer thioglycollate (BT) on the adherent cell population of the peritoneal cavity

Group lavage		Cell recovery ($\times 10^5$/mouse) Neutrophils	Macrophages
Controls			
BALB/c	Pre-trypsin	0·4 ± 0·08	2·9 ± 0·72
	Post-trypsin	0·1 ± 0·02	1·6 ± 0·40
C57Bl/10	Pre-trypsin	0·1 ± 0·04	4·6 ± 0·60
	Post-trypsin	0·1 ± 0·04	0·6 ± 0·26
18 h BT			
BALB/c	Pre-trypsin	7·5 ± 2·60	4·1 ± 1·64
	Post-trypsin	3·3 ± 0·34	2·8 ± 0·87
C57Bl/10	Pre-trypsin	35·9 ± 7·20	8·6 ± 0·96
	Post-trypsin	5·9 ± 0·84	3·2 ± 0·85

Mice were given 4 ml peritoneal lavage before or 18 h after injection of 1 ml 3% BT. The peritoneal cavity was then flushed with 4 ml buffer and incubated for 10 min at 37°C with 4 ml warm 0·25% trypsin in PBS. The trypsin solution was withdrawn, reinjected forcibly to remove loose cells, re-aspirated, then mixed with equal volumes of ice-cold RPMI + 10% FCS. When PBS was substituted for trypsin, less than 104 total cells/mouse were recovered.

phenotype for both neutrophils and macrophages, whereas casein and *Staph. aureus* induced a LR phenotype for both cell types. In contrast, *Staph. epidermidis* induced high macrophage but low neutrophil responses. In F_1 mice LPS stimulation induced the HR phenotype for both cell types.

Adoptive transfer of peritoneal inflammatory cells

The inflammatory status of the cell donor (naive or 12 h p.i. with BT) did not significantly influence the subsequent response in the recipient. Mean neutrophil and macrophage numbers in recipients at 18 h p.i. were $6{\cdot}0 \pm 3{\cdot}1 \times 10^5$ and $5{\cdot}0 \pm 2{\cdot}4 \times 10^5$ (naive cells) and $3{\cdot}2 \pm 1{\cdot}3 \times 10^5$ and $3{\cdot}7 \pm 1{\cdot}2 \times 10^5$ (stimulated cells). In animals injected with saline only, recoveries were $0{\cdot}16 \pm 0{\cdot}08 \times 10^5$ neutrophils and $10{\cdot}8 \pm 2{\cdot}5 \times 10^5$ macrophages/mouse. The magnitude of the neutrophil response to injected cells was approximately 4-fold lower than to BT.

Effect of exogenous IL-1α or TNF-α (Table 3)

The i.p. inoculation of IL-1α resulted in a neutrophil response at 18 h p.i. in both strains which was within the range known to be induced by BT in BALB/c mice [8], but a macrophage response comparable to B.10 mice (see Fig. 1d). In contrast, the response to TNF-α was strain-dependent. Neutrophil numbers were minimal in BALB/c but comparable in B.10 mice to levels induced by BT. Both strains showed a HR macrophage phenotype.

DISCUSSION

Mice show genetically determined variation in the acute peritoneal neutrophilia elicited by i.p. injection of a sterile irritant [8]. This variation could reflect strain-dependent differences in the kinetics and/or cytokine production of three major transitory cell populations within the peritoneal cavity – T cells, macrophages and mast cells – all of which could potentially regulate neutrophil influx.

Although T cells have been implicated in mediating genetic variation in macrophage and neutrophil-mediated resistance to many infections, these effects are associated more with chronic than acute neutrophil accumulation [18] and T cell-independent mechanisms may operate simultaneously [19]. Peritoneal lymphocytes increased only slightly during the first 18 h after stimulation and levels of T cell cytokines in peritoneal exudate fluid changed little over 24 h. IL-3 values were low throughout, IFN-γ showed only a small increase initially and GM-CSF was not detectable at any time.

Mast cells have large intra-cellular stores of mediators that can be released when the cells degranulate following stimulation [16,20,21]; some of these mediators, notably TNF and IL-1, can effect neutrophil accumulation. The abrupt decline in numbers of mast cells during the first 9 h p.i. following injection of BT suggests rapid degranulation, but the kinetic pattern of response was essentially similar in the two strains during the critical 12–18 h period.

Macrophage numbers recovered by peritoneal washing also declined in the first 9 h p.i. in both strains but then increased, the rise occurring earlier in B.10 mice. The changes in proportions of adherent/non-adherent cells suggest that the initial decline reflects adherence, not emigration, the subsequent increase reflecting immigration. Adherent macrophages are likely to be activated and thus capable of releasing regulatory mediators that may initiate neutrophil accumulation. However, neutrophil numbers continued to rise in B.10 mice and this rise coincided with the increase in macrophages, suggesting a functional relationship. If correct, response phenotypes for the two cells should be correlated whatever the eliciting stimulus and co-inherited. Indeed, the high neutrophil and macrophage responses were seen in B.10 mice after injection of BT, killed Gram-negative bacteria or LPS, removal of LPS from BT abrogating both responses. F_1 (BALB/c ×C57Bl/10) hybrids showed both high neutrophil and high macrophage responses when injected with BT. Non-LPS mediated stimuli (casein, killed *Staph. aureus*) induced low macrophage and neutrophil responses. There is, therefore, a suggestive correlation, but this is, unfortunately, not supported by the cytokine data. GM-CSF and TNF were not detectable at any time, whilst G-CSF showed a comparable response in both B.10 and BALB/c with an early peak at 3 h p.i. followed by a steady decline. In addition, the neutrophil–macrophage response correlation was not seen in response to *Staph. epidermidis*. These data, together with the adoptive transfer data, suggest that the genetic control of the neutrophil response after LPS stimulation is not dependent upon the accompanying macrophage influx.

This study focused upon inflammatory events within the peritoneal cavity itself. However, since inflammation is mediated through sequestration of effector cells from the circulation, a process in which expression of adhesion molecules plays a key role, genetically determined variation in peritoneal neutrophil accumulation may be mediated through variation in pro-inflammatory cytokines such IL-1 and TNF via their effects upon expression of adhesion molecules on endothelial cells. After BT stimulation, both strains produced IL-1α from 3 h p.i. and both showed a comparable neutrophil influx when injected with IL-1α. In contrast, TNF-α induced a strain-dependent neutrophil accumulation comparable to that induced by BT. Although LPS triggers TNF release from murine peritoneal macrophages *in vitro* [22,23], no TNF-α was detected in peritoneal exudate or blood from animals of either strain during the first 24 h of a BT-induced inflammatory

Table 3. Effect of exogenous IL-1α or TNF-α on peritoneal inflammatory response: IL-1α or TNF-α were injected i.p. and peritoneal cells harvested after 18 h; counts are expressed as the mean of either five or six replicate mice ± 1 s.e.

Treatment	Strain	Cell count ($\times 10^5$/mouse) after 18 h: Neutrophils	Macrophages
Control	BALB/c	$0{\cdot}16 \pm 0{\cdot}12$	$16{\cdot}4 \pm 3{\cdot}6$
	C57Bl/10	$0{\cdot}36 \pm 0{\cdot}20$	$12{\cdot}4 \pm 2{\cdot}4$
500 ng TNF-α	BALB/c	$5{\cdot}1 \pm 2{\cdot}4$	$16{\cdot}4 \pm 6{\cdot}0$
	C57Bl/10	$40{\cdot}4 \pm 14{\cdot}8$	$27{\cdot}2 \pm 6{\cdot}4$
		$P < 0{\cdot}05$	$P = 0{\cdot}27$
50 ng IL-1α	BALB/c	$17{\cdot}2 \pm 3{\cdot}3$	$14{\cdot}4 \pm 3{\cdot}5$
	C57Bl/10	$25{\cdot}1 \pm 5{\cdot}5$	$13{\cdot}1 \pm 1{\cdot}5$
		$P = 0{\cdot}27$	$P = 0{\cdot}5$

Data were compared using the Mann–Whitney *U*-test.

response. Nakane *et al.* [24] reported comparable findings in *Listeria*-infected mice where, despite the fact that no TNF-α was detected (L929 bioassay, $< 4\,\mu$/ml) in serum or spleen homogenates at any time point, injection of animals with anti-TNF-α antibody 0–2 h p.i. significantly reduced survival. Failure to detect TNF-α *in vivo* may reflect its very short half-life [25], it may be active in a membrane-bound form, e.g. on endothelial cells, or it may operate locally over concentration gradients not detectable in crude peritoneal washings.

These experiments have analysed genetically determined differences in the LPS-induced accumulation of neutrophils at a site of acute inflammation. A number of possible explanations for such differences have been excluded and attention is now focused on variation in expression of endothelial adhesion molecules as a contributory mechanism. *In vivo* blocking of selected adhesion molecules by injection of specific antibody should determine their role in the greater accumulation of neutrophils seen in in high-responders.

ACKNOWLEDGMENTS

This work was supported by a grant from the Wellcome Trust. Staphylococci were kindly supplied by Professor R. G. Finch, City Hospital, Nottingham and *E. coli* by Dr P. Tighe, Department of Microbiology.

REFERENCES

1 Shaper AG, Lewis P. Genetic neutropenia in people of African origin. Lancet 1971; **i**:1021–4.

2 Fletcher J, Haynes AP, Crouch SM. Acquired abnormalities of polymorphonuclear neutrophil function. Blood Rev 1990; **4**:103–5.

3 Stolc V. Genetic control of blood neutrophil concentration in the rat. J Immunogenet 1988; **15**:345–9.

4 Kissling K, Karbe E, Freitas EK. *In vitro* phagocytic activity of neutrophils of various cattle breeds with and without *Trypanosoma congolense* infection. Tropenmed Parasit 1982; **33**:158–3.

5 Sluiter W, Elzenga-Claasen I, Oomens LWM *et al.* Differences between B10 and CBA mice in the regulation of the granulocyte and macrophage inflammatory response. Progr Leuk Biol 1985; **3**:567–70.

6 Czuprynski CJ, Canono BP, Henson PM, Campbell PA. Genetically determined resistance to listeriosis is associated with increased accumulation of inflammatory neutrophils and macrophages which have enhanced listericidal activity. Immunology 1985b; **55**:511–4.

7 Ali NMH, Behnke JM, Manger BR. The pattern of peripheral blood leucocyte changes in mice infected with *Nematospiroides dubius*. J Helminth 1985; **59**:83–8.

8 Marley SB, Hadley CL, Wakelin D. Genetic variation of induced neutrophilia in mice. Infect Immun 1994; **62**:4304–9.

9 Metcalf D. Hematopoietic regulators: redundancy or subtlety? Blood 1993; **82**:3515–23.

10 Furie MB, McHugh DD. Migration of neutrophils across endothelian monolayers is stimulated by treatment of the monolayers with IL-1 or TNF-α. J Immunol 1989; **143**:3309–17.

11 Le J, Lin J-X, Henriksen-DeStefano D, Vilcek J. Bacterial LPS-induced IFN-γ production: roles of IL 1 and IL 2. J Immunol 1986; **136**:4525–30.

12 Doherty GM, Lange JR, Langstein HN *et al.* Evidence for interferon-γ as a mediator of the lethality of endotoxin and TNF-α. J Immunol 1992; **149**:1666–70.

13 Heinzel FP, Rerko RM, Ling P *et al.* Interleukin 12 is produced *in vivo* during endotoxemia and stimulates synthesis of gamma interferon. Infect Immun 1994; **62**:4244–9.

14 Seljelid R, Busund L-Tr. The biology of macrophages: II Inflammation and tumors. Eur J Haematol 1994; **52**:1–12.

15 Qureshi, R, Jakschik, BA. The role of mast cells in thioglycollate-induced inflammation. J Immunol 1988; **141**:2090–6.

16 Burd PR, Rogers HW, Gordon JR *et al.* Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. J Exp Med 1989; **170**:245–7.

17 Shirofuji N, Asano S, Matsuda S *et al.* A new bioassay for human granulocyte colony-stimulating factor (hG-CSF) using murine myeloblastic NFS-60 cells as targets and estimation of its level in sera from normal healthy persons and patients with infectious and hematological disorders. Exp Haematol 1989; **17**:116–9.

18 Appleberg R, Silva M. T cell-dependent chronic neutrophilia during mycobacterial infections. Clin Exp Immunol 1989; **78**:478–83.

19 Conlan JW, Sjostedt A, North RJ. CD4^{+} and CD8^{+} T-cell-dependent and -independent host defense mechanisms can operate to control and resolve primary and secondary *Francisella tularensis* LVS infection in mice. Infect Immun 1994; **62**:5603–7.

20 Dahinden CA, Brunner T, Krieger M *et al.* Cytokines in allergic inflammation. Agents Actions 1993; **43**:189–96.

21 Baggiolini M, Dahinden CA. CC chemokines in allergic inflammation. Immunol Today 1994; **15**:127–31.

22 Tesh VL, Ramegowda B, Samuel JE. Purified Shiga-like toxins induce expression of proinflammatory cytokines from murine peritoneal macrophages. Infect Immun 1994; **62**:5085–94.

23 Nakano Y, Onouka K, Terada Y *et al.* Protective effect of recombinant TNF-α in murine salmonellosis. J Immunol 1990; **144**:1935–41.

24 Nakane A, Minagawa T, Kato K. Endogenous tumour necrosis factor (Cachetin) is essential to host resistance against *Listeria monocytogenes* infection. Infect Immun 1988; **57**:2563–69.

25 MacDonald TT, Murch SH, Nicholls SW, Breese EJ. Intestinal cytokines in inflammatory bowel disease and invasive diarrhoea. Trans R Soc Trop Med Hyg 1993; **87**:Supplement 3:23–6.